

Annex C.4

Characterising Water in Coffee using Nuclear Magnetic Resonance (NMR)

1. Introduction

Water heterogeneity in equilibrated coffee beans can be characterised using nuclear magnetic resonance (NMR) and NMR imaging. The interpretation of such images is complex and not absolutely certain due to overlapping phenomena that affects apparent binding to biological macro molecules, and the possible expenditure of metabolic energy to pump water, but, in an equilibrated system, the wet areas should correspond to the hygroscopic areas.

2. Brief description of NMR experiment

The nuclear magnetic resonance (NMR) work reported here was carried out at the physics department of the University of Surrey using a non-commercial research imaging instrument with a resolution of 10 μ and a Marran bench-top instrument for the acquisition of total NMR decay signal. Neither instrument could be cooled below about 30 to 35°C so this was the operating temperature for all acquisitions. The imaging system was controlled using a Unix work station.

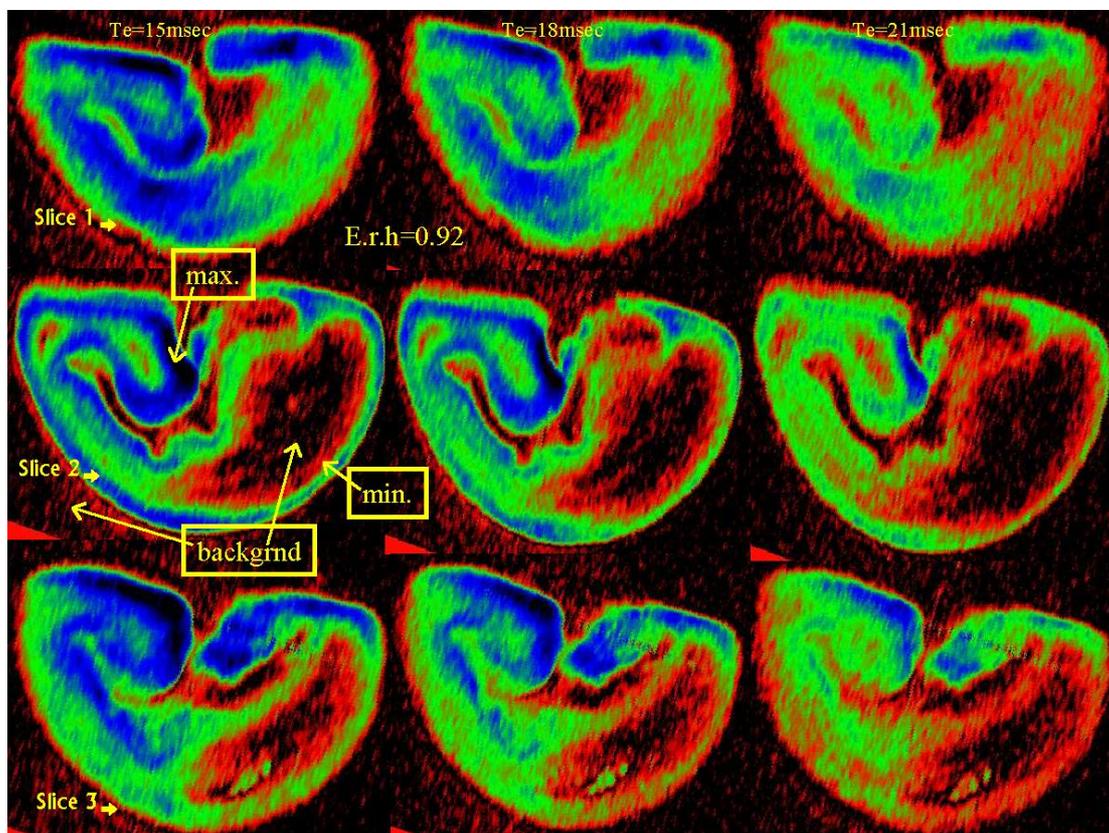
For the imaging, the beans were pre-equilibrated over H₂SO₄ solutions then maintained in equilibrium in sealed NMR tubes containing some fresh A_w controlling solution below the acquisition path. The bean was positioned on a dry cotton plug which was found to contribute negligible interference or distortion. The images, collected at a pre-set relaxation time at three to five slices required 60 to 120 minutes each, depending on specifications. Many preliminary images were required to optimise the acquisition parameters.

For total NMR signal acquisition, both pre-equilibrated, as above, beans and beans taken from a drying time-course were measured. The bench top machines required 0.5 to 5 minutes per determination, depending on the water content.

3. Findings of NMR study

The NMR image in Figure A indicates that the greatest accumulation of water is in the tissues lining the centre cut and the flat side of the seed. Note also there is always a gradation to the surface with the wettest regions following the surface but separated from it by other tissue. There is generally more water in the first slice and it is more evenly distributed. Overall there is less loosely bound water and almost no water of any kind in the centre of the cotyledons.

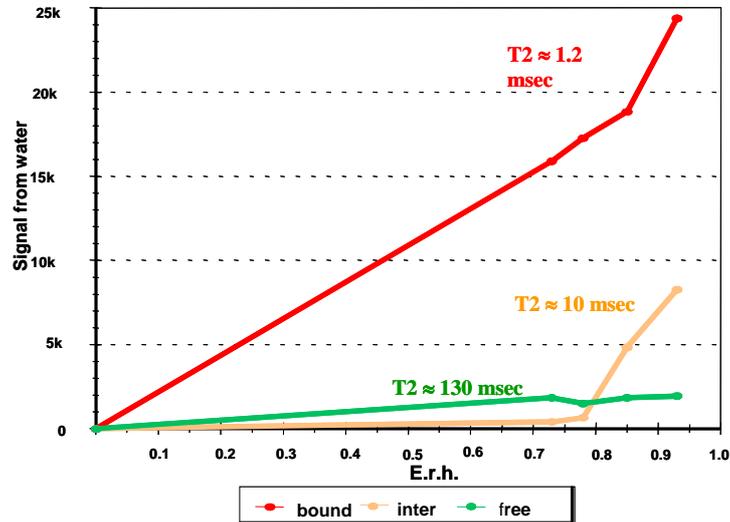
Figure A: Three optical cross-sections (top to bottom) of an equilibrated ($A_w = 0.92$) coffee bean. The images locate populations of progressively less tightly bound water ($t_2 = 15$ to 21msec) from left to right of one optical slice, while the relative intensity of the signal is calibrated to colour.



Aside from imaging in narrowly defined signal acquisition, NMR can be used to measure total populations of water as distinguished by the strength of its binding to the matrix.

Figure B shows how the different populations of water contribute to the water that is lost during drying. Down to an A_w of 0.86, water is lost equally from the bound and intermediate populations. Between this hydration level and 0.78, water is disproportionately lost from the intermediate population which is likely to be available, unlike the bound water, for fungal growth.

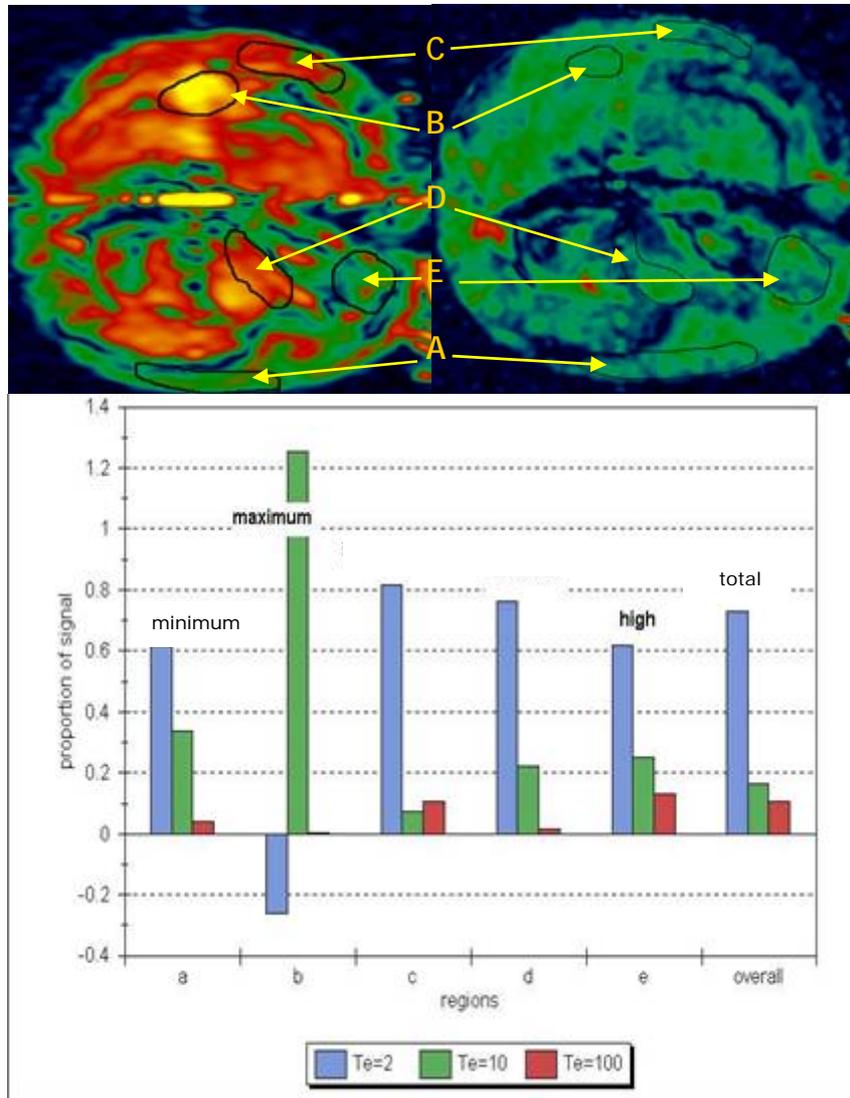
Figure B: Distribution of water populations as described by the strength of their binding to elements of the coffee bean. The '0' point is oven dried beans deducted from the total signal to account for non-water protons. All determinations are means of five equilibrated beans using H₂SO₄ solutions.



It is possible to calculate total signal from defined regions and calculate the contributions of the different water binding populations from that signal in the images.

In Figure C the signal from a longitudinal optical slice was collected from five selected regions and the signal evaluated into the three populations as defined in the work shown in Figure B. It can be seen that different regions have a different availability of water binding sites that correspond to the three populations.

Figure C: The relative contribution of water from the three identifiable binding energy populations in wet and dry regions of the seed. The selected sites, A-E, represent areas with the maximum to the minimum amount of water and the total signal from each of these was recalculated so to reveal the relative contributions of the known (see Figure B) water populations. Here, the signal is calibrated from Yellow (max) to dark blue (min). $A_w = 0.92$.



Region B above probably corresponds to the subsurface wet region near the flat surface seen in Figure A. Here there is no strongly bound water and no 'free' water. It all seems to be 'physiological' water, the intermediate population we have alluded to previously, available for microbial (and plant) growth. The driest region, A, shows slightly more intermediate water than expected, based on pattern of the total, whereas only the rather dry region 'E' has even slightly more free water than expected. Bound water is evenly distributed to all regions within 5% as is the free water so it is the intermediate water that is apparently most exchangeable as it is in comparing different states of dryness (Figure B).

The intermediate water plays a structural role in the bean as well. Figure D shows that addition of water produces a disproportionate increase in the volume of the coffee bean: it causes it to swell. The A_w range where we see this swelling

corresponds closely to the disproportionate water loss of the intermediate water population between A_w 0.87 and 0.78. In m.c. terms this corresponds to about 25 and 13% (db).

Figure D: Change of bean volume with the addition of water to dry beans. The beans had been air dried to a m.c. of 5.7% and volume measured by the displacement method. The (red) line represents the ml-for-ml or expected change in volume.

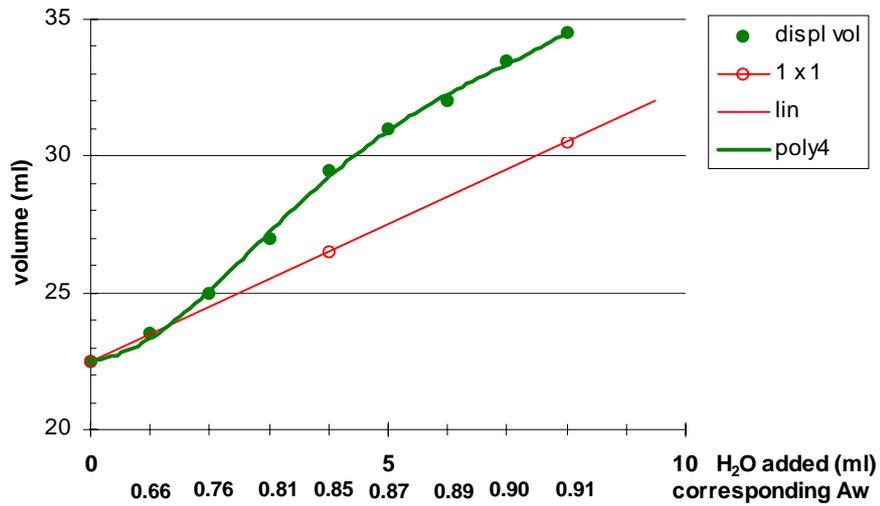
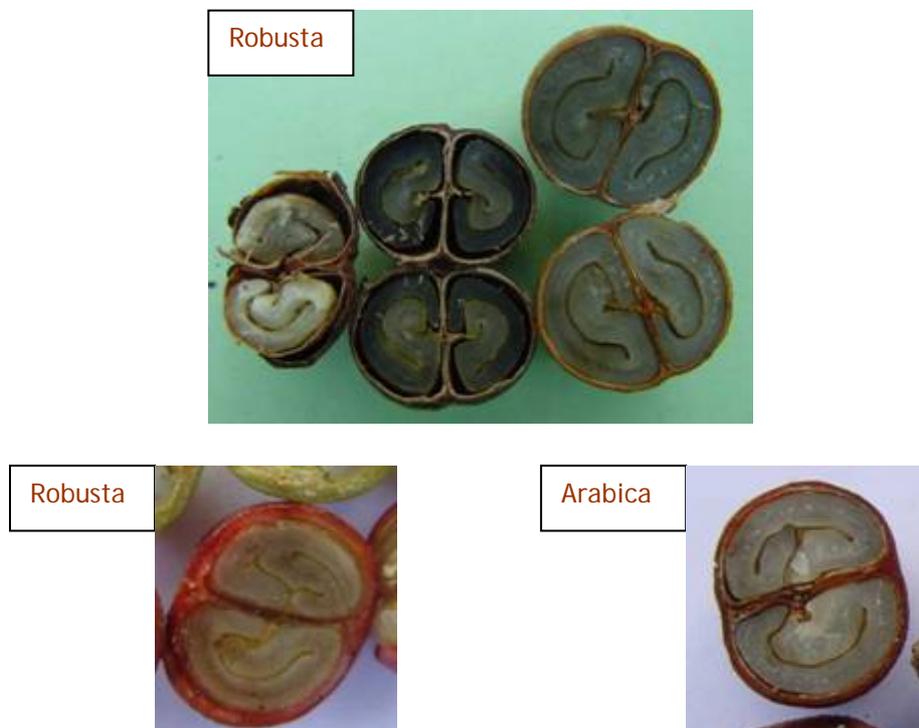


Figure E: Robusta cherry in various stages of drying, and a comparison between ripe robusta and arabica cherry showing the difference in mesocarp thickness.



Cherry coffee, with its additional tissues shows a different picture than does the bean. From Figure F it can be seen that in amount and distribution in binding populations, water in green and half-ripe cherry is more or less identical but, as full ripeness is attained, there is an increase in water content. Water loss through senescence and drying on the tree is from the component of free water. It seems likely that the bound component of water should mostly lie within the bean, probably associated with the carbohydrate-bearing cells of the cotyledons. Upon rewetting it is the more hygroscopic sites, with higher binding energy, that are first recharged. That the bound component increases dramatically could indicate that water is distributed differently in re-wetted coffee than in drying coffee, and may represent the process known to seed physiologists as 'imbibition' where water is rapidly conducted inward to re-hydrate the metabolically active tissues of the seed.

Figure F: NMR analysis conducted at 35°C. Signal amplitude measures protons almost all of which is attributable to water due to the method of measurement. The figures on the bars are the T2 values in msec and relates to binding of the protons. Based on means of three determinations.

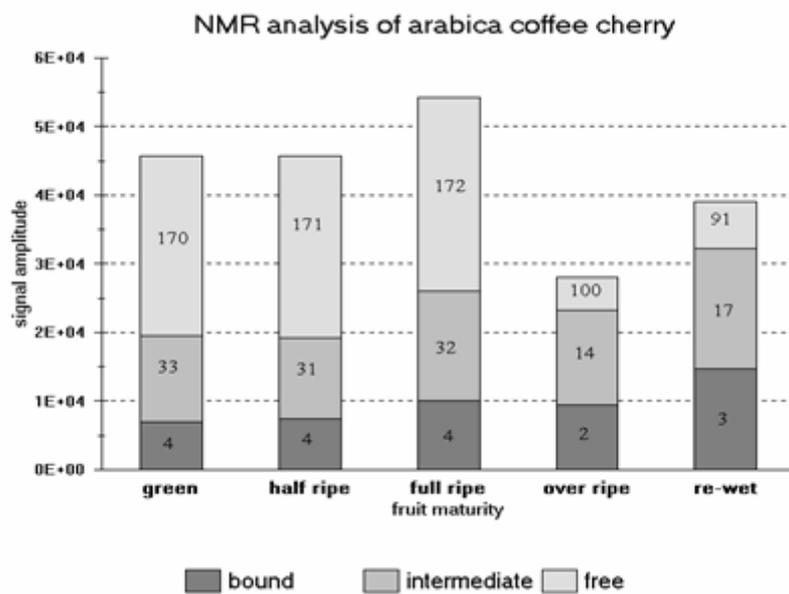
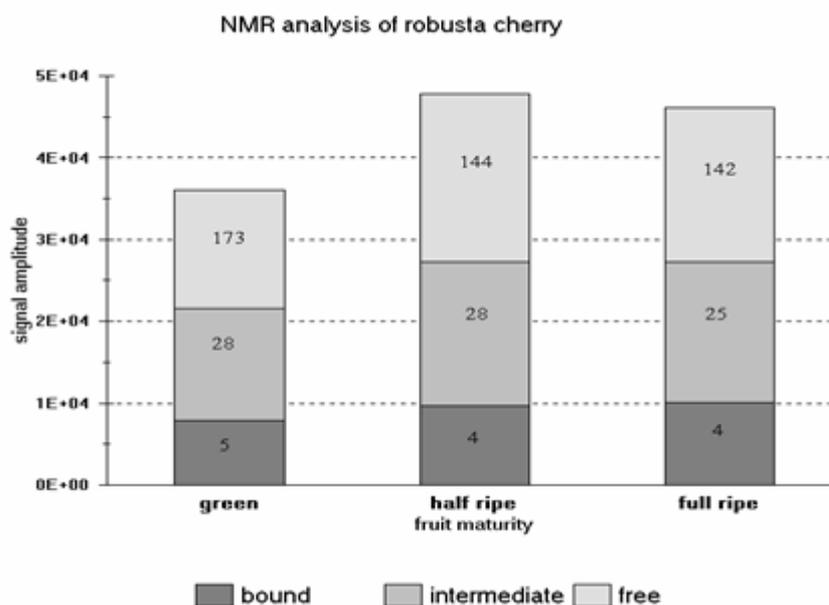


Figure G: NMR analysis conducted at 35°C. Signal amplitude measures protons almost all of which is attributable to water due to the method of measurement. The figures on the bars are the T_2 values in msec and relates to binding of the protons. Based on means of three replicates.

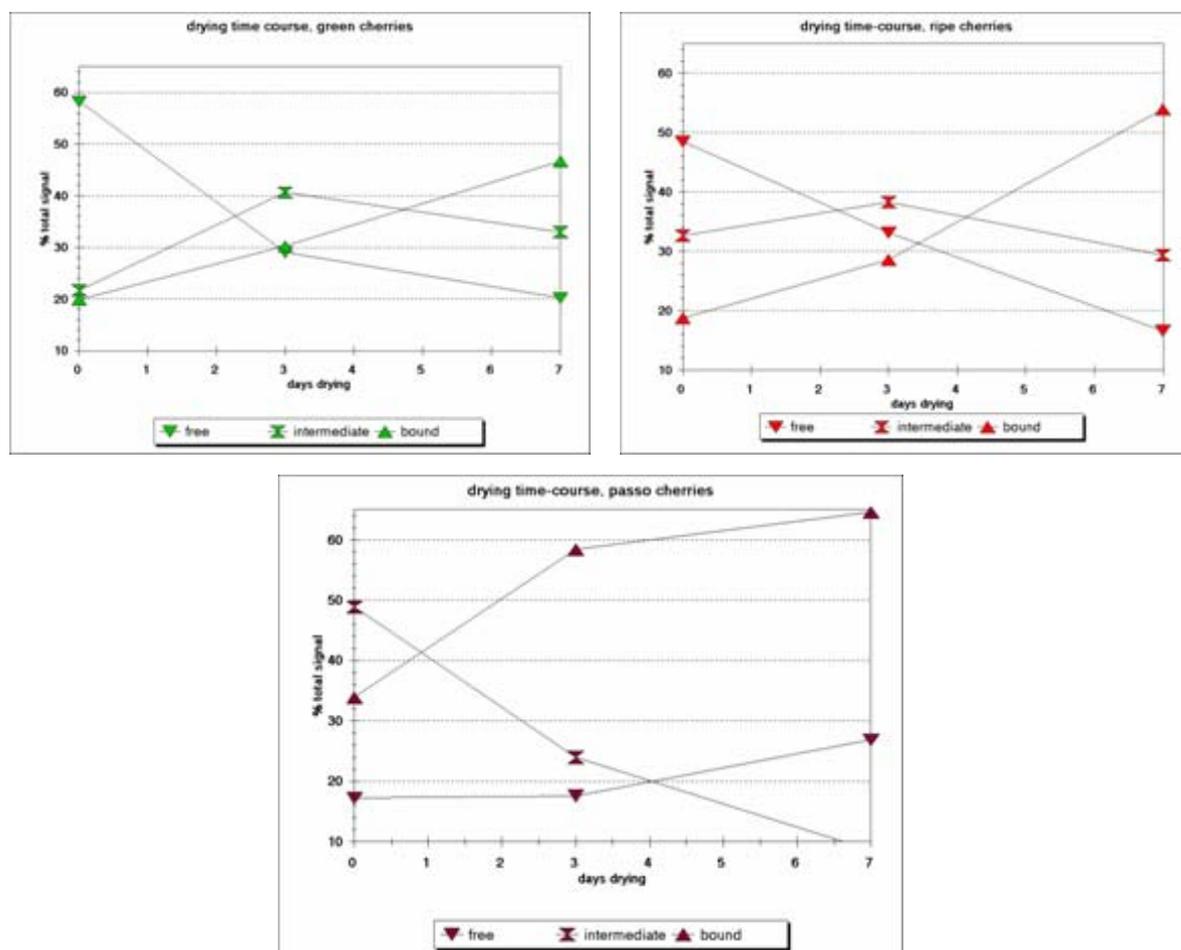


The values of T_2 in msec (the numbers printed on the bars in Figures F and G) show the degree of binding of the water populations described above as 'bound', 'intermediate' and 'free'. These figures should be understood as mean values around which there is a statistically discernible distribution separate from the other means and their distributions. These are virtually invariant through full ripeness but show a fall (more tightly bound on average) as the tissues reach senescence and dry. If most of the tightly bound water is associated with the bean tissue, rather than the fruit tissue, the observed rehydration pattern means the bean, although slower to dry, is faster to re-wet.

A comparison of the 'free-water' in robusta and arabica cherry is consistent with the free water being mostly in the mesocarp since the thinner robusta mesocarp corresponds with a smaller 'free-water' signal (Figure F). The components of free and intermediate water are of a nearly equal size and these components show a slightly higher degree of binding than do the corresponding components in arabica. Consistent with the opinion in the field that robusta is overripe when fully red, the half ripe sample most resembles fully ripe arabica in its water distribution and content. There was no over-ripe robusta sample available.

Figures H, I, and J, below, show the NMR drying time-courses (in the laboratory under a heat lamp) of green, ripe and 'passo' arabica cherry, respectively. In green and ripe cherry about half of the total water is loosely bound water ('free') in contrast to the passo in which this component comprises less than one fifth. In fact the patterns of green and ripe are comparable, with the free water falling to about one-fifth while the bound water rises to about 50% by the seventh day.

Figures H, I & J: The NMR drying time-course of green, ripe and passo cherry coffee, respectively over 7 days under a heat lamp. Three populations of water can be distinguished, referred to as free, intermediate and bound corresponding to T_2 values ranging from about 170 to 110 msec for 'free'; 40 to 12 msec for 'intermediate'; 4 to 0.5 msec for 'bound'.



The population of water subject to intermediate degrees of binding is steady at around 30% throughout the measurement period. It is interesting that *passo* cherries appear to have a fundamentally different distribution of water than cherries dried 'artificially'. As the water content falls, the bound water becomes the largest component of the total regardless of the stage of ripeness when drying began.

One of the differences between natural and wet processing is that in wet processing almost half of the water is removed *before* the coffee is presented to the drying technology.

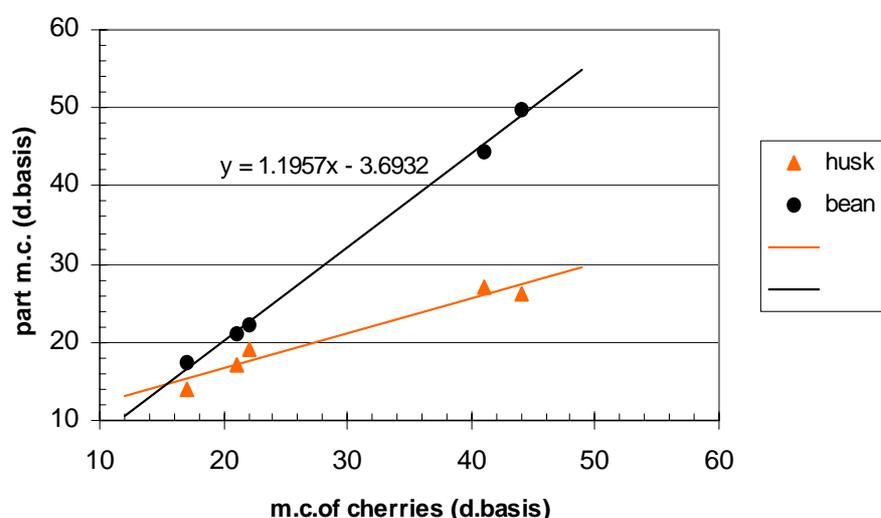
The second difference is that the properties of the fruit tissue are different to those of the bean, and while the husk provides a barrier to water loss, it also protects the bean. In addition, as shown above in Figures D and E, the bean shrinks away from the husk at around 25% m.c. causing an air-lock to form, thus further inhibiting water loss from the bean.

The oven dried m.c. of five robusta cherry samples, at various stages of drying, was determined as whole cherries, and separately as husk and beans. The proportion of dry matter in husks and beans was determined by direct, individual measurement of ten oven-dried cherries from each of the five samples. These data were used to reconstruct the distribution of water in the samples.

In Figure K it can be seen that the m.c. of the beans in partially dried cherries is higher than that of the husks. One would predict that if the relationship had extended to fresh cherries, the proportion of water in the bean would flatten and perhaps fall where the mucilage is hydrated. When the cherries were at 44% m.c., the beans were at 50% and the husk at 25%. The proportion of total water residing in the bean for the points in Figure K falls along a line from 73% in the wettest sample to 61% in the driest. Though there is a considerable difference between husk and bean m.c., the cherry m.c. is only slightly different from that of the bean.

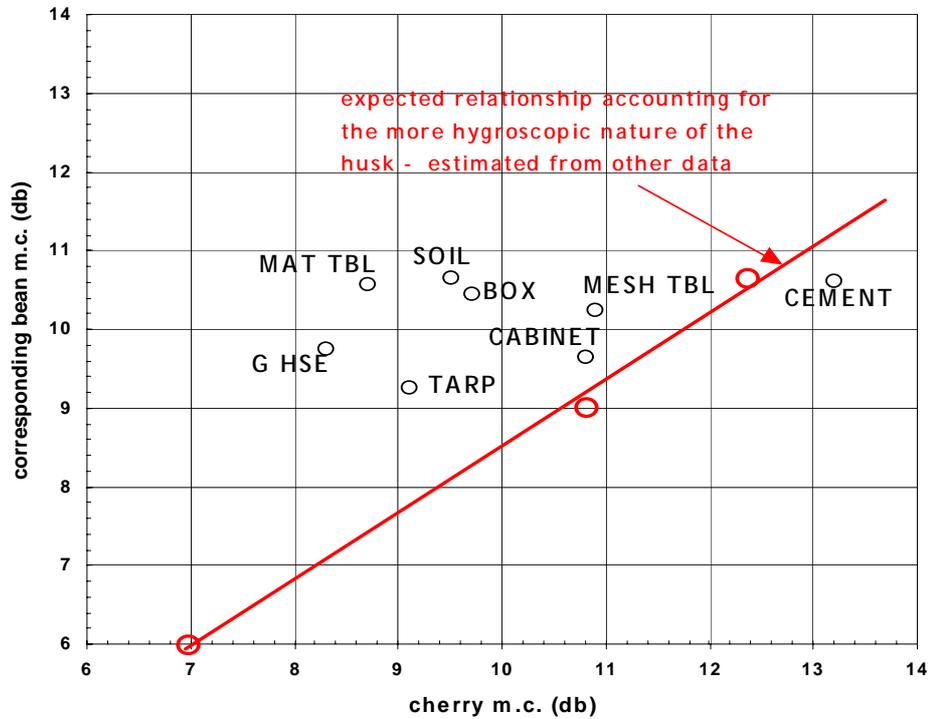
A slight compensation could be made according to the line of best fit above: $y=1.196x-3.7$. Given a cherry m.c. of 30%, this indicates that the beans are at 32.2%. Arabica is presumably qualitatively the same as robusta, discussed here, but with its relatively thick mucilage layer is likely to be quantitatively different to robusta.

Figure K: Moisture content of beans and of all other fruit tissues ('husk') of robusta cherry samples from Lampung Barat, Indonesia, that had been dried to varying degrees. This represents the disequilibrium situation of partially dried cherries.



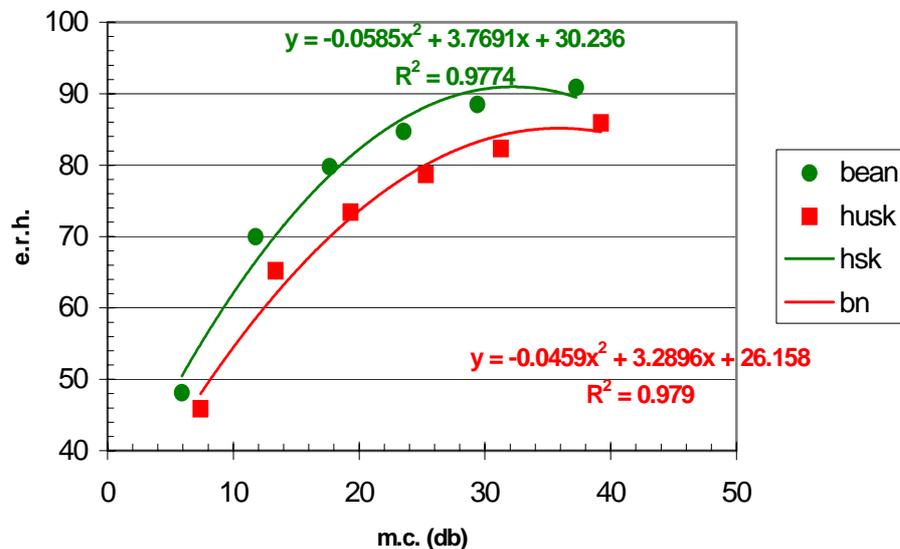
Evidence for disequilibrium in the dried product arises from the studies reported in Figure L. Most of the drying methods yielded beans which retained more than the expected proportion of water given the moisture content of the system (the cherry). The expected relationship was calculated from bean and husk sorption isotherms. The impact of this observation is probably slight with the well dried product but if the principle holds at 25% cherry moisture content, it could be significant, the more so since the A_w of the bean in this moisture range is some 0.07 higher than the corresponding A_w of husk. The context relates to poorly dried cherry coffee and the issue of dehusking poorly or partly dried coffee discussed in the section on drying yard management.

Figure L: The moisture content of dried product (cherry) and the corresponding beans after completion of the drying trials. 'BOX', 'CABINET' and 'G HSE' are three designs of solar dryers; 'MAT TBL' = drying table covered with coconut matting; 'MESH TBL' = drying table covered with wire mesh.



Throughout the moisture range tested, at a given m.c., the beans have an ERH 8-10% higher than the husk. This implies that the husk is more hydrophilic than the bean and that the water in the bean is more available for loss (or utilization). Loss from the bean to the open air, however, would be retarded by intact husk not only because it is enclosed by the husk but also because the husk binds water more tightly than does bean tissue.

Figure M: Sorption isotherms for the beans and all other fruit tissue ('husk') of Indian (Coorg) robusta cherry coffee.



Since the properties of bean and husk differ markedly, if the proportion of these components varied, this would be expected to influence the A_w /m.c. relationship. Table A shows how this proportion can vary in some common defects.

Table A: Proportion of the total cherry dry matter comprising bean tissue with certain defects.

Cherry description	Bean dry weight	Proportion of cherry	(n)
Normal	326	0.62	(33)
Degraded	176	0.44	(7)
Black	222	0.52	(3)
<i>Broca</i>	378	0.61	(2)
Peaberry	168	0.51	(3)